The following listing of claims replaces all prior versions:

**Listing of the claims**

1. **(Currently Amended)** A method ~~and kit~~ for determining the presence of bacteria or fungus-yeast ribonucleic acid (RNA) in a sample suspected of containing said bacteria and/or fungus, wherein said ~~polynucleotide~~ RNA comprises a selected target ~~region~~ sequence, said method comprising:

(a) ~~extract bacteria or fungus-yeast ribonucleic acid (RNA) from the sample up to 1000 ml by centrifiltration on membranes and /or DEAE resin following by incubation with DNAse.~~providing a sample to be tested or which is suspected of containing particular bacteria or fungus-yeast RNA.

(b) incubating the bacteria or fungus-yeast ~~ribonucleic acid~~ [[(RNA)]] RNA with a thermostable enzyme with RNA-dependent Reverse Transcriptase activity and with DNA-dependent Polymerase activity~~, allowing the combination of RT and PCR in a single tube reaction, such as Tth DNA polymerase, and polynucleotide primers with a nucleotide sequence selected from the group consisting of~~

~~Seq ID No 2 TGCGGGACTTAACCCAACA [primer reverse]~~
~~Seq ID No 4 TTACCCCACCTACTAGCTAAT [primer reverse]~~
~~Seq ID No 6 TTGCGCTCGTTRCGGGACTT [primer reverse]~~
~~Seq ID No 8 CGTTATCGCAATTAAGCAGACA [primer reverse]~~
~~Seq ID No 10 TTGGGTAATTTGCGCGCCTG [primer reverse]~~

under conditions which allow ~~hybridization of the polynucleotide to~~ the ~~ribonucleotide target region and~~ Reverse Transcriptase activity of said ~~DNA polymerase for~~thermostable enzyme to synthesize cDNA ~~synthesis~~from the RNA target sequence; ~~and~~

~~(c) amplified~~ amplifying the cDNAs formed to a detectable level by Polymerase Chain Reaction with said DNA polymerase activity of the thermostable enzyme and polynucleotide primers and; ~~probes with a nucleotide sequence selected from the group consisting of~~

(c) detecting the amplified cDNAs by hybridization with one or more probe polynucleotide(s).

~~Seq ID No 1 TGGAGCATGTGGTTTAATTCGA [primer forward]~~
~~Seq ID No 2 TGCGGGACTTAACCCAACA [primer reverse]~~
~~Seq ID No 3 AGAGTTTGATCATGGCTCAGA [primer forward]~~
~~Seq ID No 4 TTACCCCACCTACTAGCTAAT [primer reverse]~~
~~Seq ID No 5 GYGGAGCATGTGGYTTAATTCG [primer forward]~~
~~Seq ID No 6 TTGCGCTCGTTRCGGGACTT [primer reverse]~~
~~Seq ID No 7 GGGAAACTCACCAGGTCCA [primer forward]~~
~~Seq ID No 8 CGTTATCGCAATTAAGCAGACA [primer reverse]~~
~~Seq ID No 9 GGTAACGGGGAATWAGGGTTC [primer forward]~~
~~Seq ID No 10 TTGGGTAATTTGCGCGCCTG [primer reverse]~~
~~Seq ID No 11 TGCATGGYTGTCGTCAGCTCGTG [probe forward]~~
~~Seq ID No 12 GAGTGGCGGACGGGTGAGTAA [probe forward]~~
~~Seq ID No 13 ACAGGTGGTGCATGGTTGTC [probe forward]~~
~~Seq ID No 14 TCAGCTCGTGTCGTGAGATGTT [probe forward]~~
~~Seq ID No 15 ACAGGTGCTGCATGGCTGTC [probe forward]~~
~~Seq ID No 16 TCAGCTCGTGTTGTGAAATGTT [probe forward]~~
~~Seq ID No 17 AGGATTGACAGATTGAGAGCTCTT [probe forward]~~
~~Seq ID No 18 CGGAGAGGGAGCCTGAGAA [probe forward]~~
~~Seq ID No 19 CGGCTACCACATCCAAGGAA [probe forward]~~

2. **(Currently Amended)** The method ~~and kit~~ of claim 1[[,]] wherein the cDNA target sequence synthesized by Reverse Transcriptase activity of the thermostable enzyme ~~like Tth polymerase~~ is amplified by the DNA-dependent Polymerase activity of ~~DNA polymerase~~ the thermostable enzyme in the same tube by means of one step real time RT-PCR.

3. **(Currently Amended)** The method ~~and kit~~ of claim 1[[,]] wherein the ~~composition for detecting bacteria comprising a~~ polynucleotide primers and [[a]] probe ~~consisting~~consist of the sequences:

Seq ID No 1 TGGAGCATGTGGTTTAATTCGA [primer forward]
Seq ID No 2 TGCGGGACTTAACCCAACA [primer reverse]
Seq ID No 11 TGCATGGYTGTCGTCAGCTCGTG [probe forward] .

4. **(Currently Amended)** The method ~~and kit~~ of claim 1[[,]] wherein the ~~composition for detecting bacteria comprising a~~ polynucleotide primers and [[a]] probe ~~consisting~~consist of the sequences:

Seq ID No 3 AGAGTTTGATCATGGCTCAGA [primer forward]
Seq ID No 4 TTACCCCACCTACTAGCTAAT [primer reverse]

Seq ID No 12 GAGTGGCGGACGGGTGAGTAA [probe forward] .

5. **(Currently Amended)** The method ~~and kit~~ of claim 1[[,]] wherein the ~~composition for detecting bacteria comprising a~~ polynucleotide primers and [[a]] probe ~~consisting~~ consist of the sequences:

| | | |
|---|---|---|
| Seq ID No 5 | GYGGAGCATGTGGYTTAATTCG | [primer forward] |
| Seq ID No 6 | TTGCGCTCGTTRCGGGACTT | [primer reverse] |
| Seq ID No 13 | ACAGGTGGTGCATGGTTGTC | [probe forward] |
| Seq ID No 14 | TCAGCTCGTGTCGTGAGATGTT | [probe forward] |
| Seq ID No 15 | ACAGGTGCTGCATGGCTGTC | [probe forward] |
| Seq ID No 16 | TCAGCTCGTGTTGTGAAATGTT | [probe forward] . |

6. **(Currently Amended)** The method ~~and kit~~ of claim 1[[,]] wherein the ~~composition for detecting fungus yeast comprising a~~ polynucleotide primers and [[a]] probe ~~consisting~~consist of the sequences:

| | | |
|---|---|---|
| Seq ID No 7 | GGGAAACTCACCAGGTCCA | [primer forward] |
| Seq ID No 8 | CGTTATCGCAATTAAGCAGACA | [primer reverse] |
| Seq ID No 17 | AGGATTGACAGATTGAGAGCTCTT | [probe forward] . |

7. **(Currently Amended)** The method ~~and kit~~ of claim 1[[,]] wherein the ~~composition for detecting fungus yeast comprising a~~ polynucleotide primers and [[a]] probe ~~consisting~~consist of the sequences:

| | | |
|---|---|---|
| Seq ID No 9 | GGTAACGGGGAATWAGGGTTC | [primer forward] |
| Seq ID No 10 | TTGGGTAATTTGCGCGCCTG | [primer reverse] |
| Seq ID No 18 | CGGAGAGGGAGCCTGAGAA | [probe forward] |
| Seq ID No 19 | CGGCTACCACATCCAAGGAA | [probe forward] . |

8. **(Currently Amended)** The method ~~and kit~~ of ~~one~~ ~~claims~~claim 1[[,]] wherein the ~~preferred combination of~~ primers and probes ~~used for detection all bacteria and/or fungus yeast consisting~~consist of the sequences:

~~Seq ID No 1+ Seq ID No 2 +Seq ID No 11~~

~~or~~

~~Seq ID No 3+ Seq ID No 4 +Seq ID No 12~~

~~or~~

~~Seq ID No 5+ Seq ID No 6 +Seq ID No 13 + Seq ID No 14 + Seq ID No 15 +Seq ID No 16~~

~~or~~

~~Seq ID No 7+ Seq ID No 8 +Seq ID No 17~~

~~or~~

~~Seq ID No 9+ Seq ID No 10 +Seq ID No 18 + Seq ID No 19~~

~~or~~

Seq ID No 1+ Seq ID No 2 +Seq ID No 11 + Seq ID No 7+ Seq ID No 8 +Seq ID No 17

or

Seq ID No 3+ Seq ID No 4 +Seq ID No 12 + Seq ID No 7+ Seq ID No 8 +Seq ID No 17

or

Seq ID No 5+ Seq ID No 6 +Seq ID No 13 + Seq ID No 14 + Seq ID No 15 +Seq ID No 16 + Seq ID No 9+Seq ID No 10 +Seq ID No 18 +Seq ID No 19.

9. **(Currently Amended)** The method ~~and kit~~ of ~~one of claims~~claim 1 [[to 8,]] wherein the polynucleotide primers and probes are natural nucleic acid or Peptide Nucleic Acid (PNA) which can hybridize to nucleic acid (DNA and RNA).

10. **(Currently Amended)** The method ~~and kit~~ of ~~one of claims~~claim 1 [[to 9]], ~~and also quantified this~~further comprising the step of quantifying the RNA ~~for~~ aby comparison with a quantified external standard RNA from ~~by example~~the group consisting of : *Escherichia coli* and *Candida spp.*

11. **(New)** The method of claims 1 or 2 wherein step (a) comprises extracting bacteria or fungus-yeast RNA from the sample up to 1000ml by contrifiltration on membranes and/or DEAE resin followed by incubation with DNAse.

12. **(New)** The method of any one of claims 1 to 3 wherein steps (b) and (c) are performed simultaneously.

13. **(New)** The method of any one of claims 1 to 4 wherein the thermostable enzyme is *Tth* DNA polymerase.

14. (New) The method of any one of claims 1 to 5 wherein the polynucleotide primers comprise: (i) a polynucleotide primer or polynucleotide primers for synthesizing cDNA by Reverse Transcription; (ii) polynucleotide primers for amplifying cDNA by Polymerase Chain Reaction; and (iii) a polynucleotide probe or polynucleotide probes for detecting the amplified cDNAs.

15. (New) The method of claim 14 wherein the polynucleotide primer(s) for synthesizing cDNA by Reverse Transcription are selected from the group consisting of:

| | | |
|---|---|---|
| Seq ID No 2 | TGCGGGACTTAACCCAACA | [primer reverse] |
| Seq ID No 4 | TTACCCCACCTACTAGCTAAT | [primer reverse] |
| Seq ID No 6 | TTGCGCTCGTTRCGGGACTT | [primer reverse] |
| Seq ID No 8 | CGTTATCGCAATTAAGCAGACA | [primer reverse] |
| Seq ID No 10 | TTGGGTAATTTGCGCGCCTG | [primer reverse] . |

16. (New) The method of claim 14 wherein the polynucleotide primers for amplifying cDNA by Polymerase Chain Reaction are selected from the group consisting of:

| | | |
|---|---|---|
| Seq ID No 1 | TGGAGCATGTGGTTTAATTCGA | [primer forward] |
| Seq ID No 2 | TGCGGGACTTAACCCAACA | [primer forward] |
| Seq ID No 3 | AGAGTTTGATCATGGCTCAGA | [primer forward] |
| Seq ID No 4 | TTACCCCACCTACTAGCTAAT | [primer forward] |
| Seq ID No 5 | GYGGAGCATGTGGYTTAATTCG | [primer forward] |
| Seq ID No 6 | TTGCGCTCGTTRCGGGACTT | [primer forward] |
| Seq ID No 7 | GGGAAACTCACCAGGTCCA | [primer forward] |
| Seq ID No 8 | CGTTATCGCAATTAAGCAGACA | [primer forward] |
| Seq ID No 9 | GGTAACGGGGAATWAGGGTTC | [primer forward] |
| Seq ID No 10 | TTGGGTAATTTGCGCGCCTG | [primer forward] . |

17. (New) The method of claim 14 wherein the polynucleotide probe or polynucleotide probes for detecting the amplified cDNAs is/are selected from the group consisting of:

| | | |
|---|---|---|
| Seq ID No 11 | TGCATGGYTGTCGTCAGCTCGTG | [probe forward] |
| Seq ID No 12 | GAGTGGCGGACGGGTGAGTAA | [probe forward] |
| Seq ID No 13 | ACAGGTGGTGCATGGTTGTC | [probe forward] |
| Seq ID No 14 | TCAGCTCGTGTCGTGAGATGTT | [probe forward] |
| Seq ID No 15 | ACAGGTGCTGCATGGCTGTC | [probe forward] |
| Seq ID No 16 | TCAGCTCGTGTTGTGAAATGTT | [probe forward] |
| Seq ID No 17 | AGGATTGACAGATTGAGAGCTCTT | [probe forward] |

Seq ID No 18 CGGAGAGGGAGCCTGAGAA [probe forward]
Seq ID No 19 CGGCTACCACATCCAAGGAA [probe forward] .

18. **(New)** The method of claim 9 wherein the polynucleotide probes further compromise a non-radioactive label.

19. **(New)** The method of claim 18 wherein the non-radioactive label is a fluoroscein.

20. **(New)** A kit for determining the presence of bacteria or fungus-yeast ribonucleic acid (RNA) in a sample suspected of containing said bacteria and/or fungus comprising:

(a) a thermostable enzyme with RNA-dependent Reverse Transcriptase activity and with DNA-dependent Polymerase activity;
(b) polynucleotide primers comprising:
  (i) a polynucleotide primer or polynucleotide primers for synthesizing cDNA by Reverse Transcription;
  (ii) polynucleotide primers for amplifying cDNA by Polymerase Chain Reaction; and
  (iii) a polynucleotide probe or polynucleotide probes for detecting the amplified cDNAs.

21. **(New)** The kit of claim 20 further comprising centrifiltration membranes and/or DEAE resin for obtaining bacteria or fungus-yeast RNA from a sample.

22. **(New)** The kit of claim 20 further comprising DNAse.

23. **(New)** The kit of any one of claims 20 to 22 wherein the polynucleotide primers for synthesizing cDNA by Reverse Transcription are selected from group consisting of:

Seq ID No 2 TGCGGGACTTAACCCAACA [primer reverse]
Seq ID No 4 TTACCCCACCTACTAGCTAAT [primer reverse]

| | | |
|---|---|---|
| Seq ID No 6 | TTGCGCTCGTTRCGGGACTT | [primer reverse] |
| Seq ID No 8 | CGTTATCGCAATTAAGCAGACA | [primer reverse] |
| Seq ID No 10 | TTGGGTAATTTGCGCGCCTG | [primer reverse] . |

24. **(New)** The kit of any one of claims 20 to 22 wherein the polynucleotide primers for amplifying cDNA by Polymerase Chain Reaction are selected from the group consisting of:

| | | |
|---|---|---|
| Seq ID No 1 | TGGAGCATGTGGTTTAATTCGA | [primer forward] |
| Seq ID No 2 | TGCGGGACTTAACCCAACA | [primer forward] |
| Seq ID No 3 | AGAGTTTGATCATGGCTCAGA | [primer forward] |
| Seq ID No 4 | TTACCCCACCTACTAGCTAAT | [primer forward] |
| Seq ID No 5 | GYGGAGCATGTGGYTTAATTCG | [primer forward] |
| Seq ID No 6 | TTGCGCTCGTTRCGGGACTT | [primer forward] |
| Seq ID No 7 | GGGAAACTCACCAGGTCCA | [primer forward] |
| Seq ID No 8 | CGTTATCGCAATTAAGCAGACA | [primer forward] |
| Seq ID No 9 | GGTAACGGGGAATWAGGGTTC | [primer forward] |
| Seq ID No 10 | TTGGGTAATTTGCGCGCCTG | [primer forward] . |

25. **(New)** The kit of any one of claims 20 to 22 wherein the polynucleotide probe or polynucleotide probes for detecting the amplified cDNAs is/are selected from the group consisting of:

| | | |
|---|---|---|
| Seq ID No 11 | TGCATGGYTGTCGTCAGCTCGTG | [probe forward] |
| Seq ID No 12 | GAGTGGCGGACGGGTGAGTAA | [probe forward] |
| Seq ID No 13 | ACAGGTGGTGCATGGTTGTC | [probe forward] |
| Seq ID No 14 | TCAGCTCGTGTCGTGAGATGTT | [probe forward] |
| Seq ID No 15 | ACAGGTGCTGCATGGCTGTC | [probe forward] |
| Seq ID No 16 | TCAGCTCGTGTTGTGAAATGTT | [probe forward] |
| Seq ID No 17 | AGGATTGACAGATTGAGAGCTCTT | [probe forward] |
| Seq ID No 18 | CGGAGAGGGAGCCTGAGAA | [probe forward] |
| Seq ID No 19 | CGGCTACCACATCCAAGGAA | [probe forward] . |

26. **(New)** The kit of any one of claims 20 to 22 wherein the thermostable enzyme is Tth DNA polymerase.

27. **(New)** The kit of any one of claims 20 to 22 for performing a method as defined in Claim 1.

28. **(New)** A method for determining the presence of bacteria or fungus-yeast ribonucleic acid (RNA) in a sample suspected of containing said bacteria and/or fungus, wherein said RNA comprises a selected target sequence, said method

comprising:

(a) providing a sample to be tested or which is suspected of containing bacteria or fungus-yeast RNA;

(b) incubating the bacteria or fungus-yeast RNA with an enzyme with RNA-dependent Reverse Transcriptase activity under conditions that allow said enzyme to synthesize cDNA from the RNA target sequence;

(c) amplifying the cDNAs formed to a detectable level by Polymerase Chain Reaction with a thermostable enzyme with DNA-dependent Polymerase activity and polynucleotide primers; and

(d) detecting the amplified cDNAs by hybridization with one or more probe polynucleotide(s).

29. **(New)** The method of claim 28 wherein the cDNA target sequence synthesized with the enzyme with RNA-dependent Reverse Transcriptase activity is amplified by the thermostable enzyme with DNA-dependent Polymerase activity in the same tube by means of one step real time RT-PCR.